

Real-time Study of Interaction Between Adenosine Triphosphate and Its Aptamer Using Dual Polarization Interferometry



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Abstract: In this work, a dual-polarization interferometry (DPI) was used to explore the binding events between adenosine triphosphate (ATP) and ATP-binding aptamer (ABA) at solid-liquid interface. The single-stranded ABA was immobilized onto the chip surface. After the addition of ATP, the real-time and label-free technique for detailed investigation of their interactions were reflected on the changes of the mass, thickness, and density through DPI. By analysis of the binding curves from changes in mass, the association rate constant (k_a , $4.66 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$), dissociation rate constant (k_d , 0.0170 s^{-1}), dissociation constant (K_A , $2.7 \times 10^5 \text{ M}^{-1}$), and association constant (K_D , $3.7 \times 10^{-6} \text{ M}$) were precisely determined. Moreover, good linear correlations between ATP concentrations and three parameters (mass, thickness, density) resolved by the response to ATP binding were obtained. The detection limits (LOD, 3σ) were $0.22 \mu\text{M}$ for mass calibration, $0.14 \mu\text{M}$ for thickness calibration, and $0.32 \mu\text{M}$ for density calibration. We expect that this DPI-based aptasensor can be utilized to study the interactions of functional ABA with ATP, and can be also used for the detection of ATP with high sensitivity.

Key Words: Dual polarization interferometry; Aptamer; Adenosine triphosphate; Interaction

1 Introduction

Aptamers, screened by an in vitro selection process termed systematic evolution of ligands by exponential enrichment (SELEX), are a class of small, single-stranded RNA or DNA nucleic acids with unique structures that can recognize and bind their cognate targets with high specificity and affinity^[1]. Compared with antibodies, aptamers possess many advantages such as excellent stability, simple synthesis and easy modification. Owing to these outstanding features, aptamers exhibit great promise in analytical, diagnostic and therapeutic applications^[2–4]. For the purpose of utilizing aptamers into research and clinical applications, it is crucial to thoroughly understand aptamer-target binding process. In this regard, a wide variety of techniques were developed for reliable

measurements, such as high performance liquid chromatography (HPLC)^[5], atomic force microscopy (AFM)^[6], capillary electrophoresis (CE) separation^[7], polymerase chain reaction (PCR) with radio detection^[8], isothermal titration calorimetry (ITC)^[9], and fluorescence assay^[10]. Despite some merits of such methods, they were suffering from several drawbacks. On one hand, direct mixing of a ligand or a target molecule with the receptor could affect their structure and the further specific binding events, leading to reduced sensitivity or selectivity. On the other hand, most of these techniques were not able to simultaneously provide real-time detailed parameters of binding process. In recent years, surface plasmon resonance (SPR) technique was widely used to investigate the interactions of protein-protein^[11], aptamer-ligand^[12], protein-metal ion^[13], etc in real time, and provide

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real-time kinetic information. However, SPR was used to study the interaction by measuring the change of refraction index, which reflected the mass changes during the interaction process, but the information about the change of conformation could not be obtained.

Very recently, dual-polarization interferometry (DPI) has become one of the most effective and powerful label-free approach for monitoring real-time data of quantitative analysis of biomolecules on interface or biomolecule interactions^[13–18]. More importantly, taking advantages of untagged reagents and offering real-time measurement data every 20 ms with high resolution of mass (0.1 pg mm^{-2}), thickness (0.01 nm) and density, DPI enables to monitor binding events on the sensing layer^[14,15]. Thus, it was extensively employed for exploring biomolecule interactions and detection applications, such as DNA immobilization and hybridization events, interactions between DNA/protein and small molecules, conformational changes of protein interactions^[16,17]. In addition, our group utilized DPI for real-time study of DNA structural changes by focusing on their interactions with small molecules such as mitoxantrone (MTX)^[18], and for the detection of thrombin using its corresponding aptamer^[19]. Consequently, DPI is regarded as a good candidate to explore the biomolecular interactions, by which the important advancements in terms of quantifying and understanding recognition events between DNA and small molecules in real time can be acquired in detail.

Adenosine triphosphate (ATP) plays an important role in cellular metabolism, and provides energy for many biochemical processes including DNA replication, bio-synthesis, adjustment of hormone and neuron activity^[20]. Abnormal level of ATP is believed to be related to cardiovascular disease, Parkinson's disease and Alzheimer disease^[21]. Detailed investigation of the interaction between ATP and its aptamer and precise determination of ATP is very important to understand the metabolism of human body and prevent the occurrence of diseases. In this work, DPI was employed to investigate interaction between ATP and its corresponding aptamer (ABA, 5'-ACC TGG GGG AGT ATT GCG GAG GAA GGT-3')^[22]. By virtue of DPI, several real-time binding information of mass, thickness and density between ATP and ABA could be simultaneously collected. The robust parameters given by DPI during the whole binding process were directly employed to further understand the interaction between ATP and ABA, where the association rate and dissociation rate of ATP-ABA were precisely determined based on the real-time mass change during the interaction process. Especially, the constructed aptasensor interface strategy was capable of offering high sensitivity for the detection of ATP and could be expected to promote the exploitation of aptasensor based on DPI for detecting target molecules in biochemical and biomedical studies.

2 Experimental

2.1 Instrument and reagents

The binding process was monitored in real-time by a dual polarization interferometer using amino-modified silicon oxynitride AnaChip (AnalLight Bio200, Farfield Sensors Ltd., Manchester, UK).

Adenosine 5'-triphosphate (ATP), guanosine 5'-triphosphate (GTP), uridine 5'-triphosphate (UTP) and cytosine 5'-triphosphate (CTP) were obtained from Sangon Biotechnology Co. Ltd. (China). Streptavidin (SA) and Glutaraldehyde (GA, 25%, *V/V*) were purchased from Sigma-Aldrich (St Louis, USA). Control DNA (cABA, biotin-5'-ACCTGTTTGAGTATTGCGGAGTAAGTT-3') and ABA (biotin-5'-ACCTGGGGGAGTATTGCGGAGGAAGGT-3') were synthesized by Sangon Biotechnology Co. Ltd. (China). Before use, oligonucleotides were dissolved in ultrapure water to 50 μM as stock solutions and were stored in refrigerator at 4 °C. Other reagents were of analytical grade and used without further purification or treatment. Ultrapure water ($18 \text{ M}\Omega\cdot\text{cm}$) from Milli-Q Synthesis (Millipore Inc., Bedford, MA) was used throughout. 20 mM Tris-HCl running buffer (150 mM NaCl, 3 mM KCl, 5 mM MgCl_2) was prepared for studying the interaction between ATP and ABA. All the buffer solutions were filtered and degassed before use. Blood samples were supplied by the Second Hospital of Jilin University. Informed consent was obtained from all patients for being included in the study. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation.

2.2 Circular dichroism characterization

Approximately 400 μL of 20 mM Tris-HCl buffer (150 mM NaCl, 3 mM KCl, 5 mM MgCl_2) containing 4 μM ABA and 15 μM ATP was added into a cell made of quartz suprasil (10 mm path length, volume 0.7 mL). The circular dichroism (CD) spectra data were collected by a JASCO J-810 spectropolarimeter (Tokyo, Japan) attached with a Peltier temperature control unit in a rectangular quartz cuvette of 10-mm path length at room temperature, with the scanning wavelength range of 210–350 nm. The scan rate was 200 nm min^{-1} . The control experiments were conducted with same procedure using cABA instead of ABA.

2.3 Immobilization of ABA

In brief, all experiments were performed at $(20 \pm 0.002) ^\circ\text{C}$ in 20 mM Tris-HCl running buffer (150 mM NaCl, 3 mM KCl, 5 mM MgCl_2). After the amino-modified chip was mounted onto the DPI instrument, the running buffer solution was flowed over the chip surface at a flow rate of $50 \mu\text{L min}^{-1}$.

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